Conformational transitions in biomolecules, especially proteins, play an important role in signaling and regulation of various biological processes. Here we propose a fast and simple method for constructing a transition pathway between two stable conformations of a protein. The protein is represented as a simplified coarse-grained (CG) model with a single site located at the C-alpha atom of each residue. The energy function of the two-state CG model is approximated by the anisotropic network model (ANM) harmonic energy surfaces of the end-states. The simple two-state energy surface comprises two local minima centered on the positions of the stable states and the system resides in one of these minima. There is a cusp with discontinuous first derivative in the multi-dimensional configuration space of the system, which acts as the transition state surface for the two-state potential. Given this simple prescription, two structures constrained to remain similar to one another but each residing on the opposite sides of the cusp, are optimized using energy minimization. This virtual ''dumbbell'' of two structures is then used as a crude approximation to the transition state. The pathway in the multi-dimensional space is constructed by letting these two structures slide down on their ANM surface using steepest descent energy minimization until the stable end-points are reached. This simple two-state ANM model was applied to explore the multi-dimensional and collective character of the conformational transition pathways in several systems of biological significance, including adenylate kinase, leucine transporter, sarcoplasmic reticulum Ca-ATPase and the glutamate transporter.

184-Plat
Structure-Based Approaches to Amyloid Inhibitors
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More than 40 human pathologies, including Alzheimer disease, are associated with protein aggregates (both amyloid oligomers and fibers). Structure-based approaches were applied for seeking amyloid inhibitors in two scenarios.
First, non-natural peptides were designed to block the fibrillation of an amyloid fiber formed by PAP248-286 (a proteolytic fragment of the protein prostate acid phosphatase), whose fiber forms enhance sexual transmission of HIV. We identified the fibril-forming segments of the PAP248-286 fiber and then determined their atomic structures. Using the structure of segments of the amyloid fiber as template, we have designed L-amino acid and non-natural L-amino acid inhibitors to binds to the end of the fibril-forming segment, blocking the addition of other segments. The 5 designed peptides and 3 non-natural peptides inhibit the PAP248-286 fibrillation and greatly inhibit the infectivity of HIV for human cells in culture.
Increasing evidence indicates soluble Aβ oligomers are the toxic species. We searched inhibitor of amyloid toxicity by characterizing binders of amyloid fiber (BAF), which stabilize amyloid fiber to reduce the toxic oligmers. The atomic structure of an amyloidogenic segment KLVFFA(Aβ16-21) binding with a BAF has been determined. Applying new modified RosettaLigand method for high-throughput docking, we identified candidate molecules from a vast space compound (~18 thousand) that interact favorably with the fiber structures. These BAF compounds bind to fibers and greatly inhibit amyloid toxicity. The derivatives of active BAF compounds were then included to expand the set. Out of 35 compounds and 24 compound derivatives tested, eight (8) unique compounds and 4 compound derivatives showed the reduction of Aβ toxicity to mammalian cells in MTT cell assays.
The success of our inhibitors demonstrated our powerful approach for structure-based inhibitor discovery, offering a new potential treatment for amyloid therapeutics.

185-Plat
Interactive Chromatin Folding, Nucleosome (Mis)Positioning and Chromatin Structure
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In the absence of the myriad influences that exist in a cell nucleus, the positioning and stability of nucleosomes on a short segment of DNA are governed by the material properties of the DNA. In the simplest view sequences of DNA that are intrinsically flexible or that are intrinsically curved lead to nucleosome positioning. Nucleosome positioning is thus an intra-nucleosome based effect. In vivo or for sequences of DNA that support multiple nucleosomes, nucleosome-nucleosome interactions and extra-nucleosomal (e.g. chromatin remodeling factors) interactions must also be considered.
In case of intra-nucleosomal based positioning, DNA material defects (defined as variations in flexibility or conformation) are hidden because the histones force the DNA to assume a well defined super helical fold. Except for specifically constructed sequences of DNA, chromatin structures determined by intra-nucleosome interactions are irregular because the nucleosome positions will be irregular.
In case inter- or extra- nucleosomal interactions determine the nucleosome positions some DNA defects will be exposed in linker DNA regions. Exposure of the defects impacts the global structure of chromatin, e.g. a bent linker yields bent chromatin.
We propose that chromatin structure is biased toward one method of positioning or the other depending on environmental conditions. Thus for a given sequence of DNA both irregular and regular structures can be obtained. Our Interactive Chromatin Modeling Web Server http://www.latech.edu/~bishop captures these ideas. Here we present a case study of how ICM Web can be used to study the folding of the mouse mammary tumor virus promoter complex(MMTV). ICM properly predicts the locations of six positioned nucleosomes in the MMTV and shows how mispositioning reveals sequence specific material defects that bend chromatin.

Platform: Muscle: Fiber and Molecular Mechanics & Structure

186-Plat
Determinants of Transversal Stiffness of Single Muscle Sarcomeres by AFM
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The titin springs of muscle sarcomeres largely determine tensile muscle stress. However, titin is incorporated into sarcomeric I-bands not strictly in parallel with the sarcomere axis, being anchored to actin at Z-disks and myosin at A-bands. On tensile sarcomere strain, which stretches the titin springs, a force component arises longitudinally, but somewhat also laterally. The latter component may contribute to increased sarcomeric transversal stiffness, decreased lateral myofilament spacing, and length-dependent activation of stretched muscle. We aimed to directly test by atomic force microscopy (AFM) force mapping whether the titin springs contribute to sarcomeric transversal stiffness and how a titin-based lateral force component would compare with other possible sources of lateral stiffness, such as osmotic forces. Single myofibrils were isolated from rabbit psoas (stiff titin-isoform) or diaphragm (compliant titin-isoform) muscles and placed in physiological buffer under the MFP-3D-BIO AFM (Asylum Research). Force curves (50x50) were performed over a region-of-interest encompassing a whole sarcomere. Force mapping revealed distinct transversal stiffness patterns along psoas and diaphragm sarcomeres, reflecting different Z-disk, I-, A-, and M-band stiffness. A-band transversal stiffness at 25nm indentation was higher in rigor (4.2pN/nm) than in relaxed sarcomeres (0.4pN/nm). Titin digestion by low-dose trypsin decreased rigor but not relaxed stiffness. A-band lateral stiffness did not differ between relaxed psoas and diaphragm sarcomeres at slack length (~2.2μm) but increased significantly after stretch to ~3.2μm, albeit more highly in psoas than in diaphragm. Following osmotic compression by 5% dextran, A-band lateral stiffness rose 5-fold, similarly in psoas and diaphragm. We conclude that stiff titin contributes more to transversal stiffness than compliant titin, confirming a role for titin in lateral force generation. However, osmotic forces alike those present *in-vivo* may laterally compress the sarcomeric lattice to a degree that the titin contribution becomes negligible.

187-Plat
Contractile Properties of Half-Sarcomeres Mechanically Isolated from Skeletal Muscle Myofibrils
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The goal of this study was to develop a system to experiment with half-sarcomeres mechanically isolated from skeletal muscle. Single myofibrils from rabbit psoas were transferred into a temperature-controlled (10°C) bath. Half-sarcomeres were isolated using two pre-calibrated glass microneedles; the first pierced externally adjacent to the Z-line, and the second internally, adjacent to the M-line. The force produced during activation of the half-sarcomere was measured by tracking the displacement of the microneedles. The half-sarcomere length (HSL) was obtained by interpolating the displacement of the needles from the initial to the final distances measured from the Z-line to the center of the sarcomere. Half-sarcomeres (n=12) produced a stress of 24.0 ±3.7 nN/um2 at HSL between 1.0 and 1.4μm. The result was comparable to that observed in isolated sarcomeres (25.5 ±3.1 nN/um2, at SL between 1.8 and 3.0μm). Preliminary trials in which we imposed stretches (ranging from 12 to 33% of the initial HSL/SL, at a speeds of